

**UNIVERSITY OF TORONTO
STUDIES**

MEDICAL RESEARCH FUND

**NO. 11: EXPERIMENTAL ENDOCARDITIS, BY H. K. DETWEILER
AND W. L. ROBINSON**

(REPRINTED FROM THE JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION, VOL. LXVII.)



**THE UNIVERSITY LIBRARY: PUBLISHED BY
THE LIBRARIAN, 1917**

University of Toronto Studies
COMMITTEE OF MANAGEMENT

Chairman: SIR ROBERT ALEXANDER FALCONER, LL.D., K.C.M.G.
President of the University

PROFESSOR W. J. ALEXANDER, PH.D.

PROFESSOR W. H. ELLIS, M.A., M.D.

PROFESSOR J. J. MACKENZIE, B.A., M.B.

PROFESSOR J. P. McMURRICH, PH.D.

PROFESSOR G. H. NEEDLER, PH.D.

PROFESSOR GEORGE M. WRONG, M.A.

General Editor: H. H. LANGTON, M.A.
Librarian of the University

EXPERIMENTAL ENDOCARDITIS

ITS PRODUCTION WITH STREPTOCOCCUS VIRIDANS OF LOW VIRULENCE

H. K. DETWEILER, M.D.

Junior Research Fellow; Instructor in Bacteriology, University of
Toronto, Faculty of Medicine

AND

W. L. ROBINSON, B.A., M.B.

Pathologist, Toronto General Hospital
TORONTO, ONTARIO

In October, 1915, there was reported from this laboratory and the department of medicine,¹ results obtained by the special methods of Rosenow in connection with an investigation into the bacteriology of the blood in a form of endocarditis less severe than the type commonly called subacute bacterial endocarditis, and in many cases milder even than those commonly recognized as simple endocarditis. We promised at that time to publish an account of the characteristics of the organisms recovered, and their action on animals. In the pursuit of this work we were hampered greatly by the special conditions made necessary by the exigencies of the war, particularly in regard to the depletion of the staff of the laboratory in response to the call to overseas service. Many phases of the work, therefore, have been allowed to remain untouched until time permits of their further investigation; but sufficient data are at hand to warrant the publication of our findings.

In following out the methods advocated by Rosenow² we have elaborated a technic which has been used for some time and which has given splendid results. Thirty c.c. of blood are withdrawn into a record syr-

1. Oille, J. A.; Graham, Duncan, and Detweiler, H. K.: Streptococcus Bacteremia in Endocarditis, *THE JOURNAL A. M. A.*, Oct. 2, 1915, p. 1159.

2. Rosenow, E. C.: The Newer Bacteriology of Various Infections as Determined by Special Methods, *THE JOURNAL A. M. A.*, Sept. 12, 1914, p. 903.

inge which has previously been sterilized and loaded with 5 c.c. of sterile 2 per cent. sodium citrate solution in normal saline. By inverting the syringe several times a thorough mixture is secured and clotting thereby prevented. The citrated blood is directly transferred to eight centrifuge tubes containing sterile distilled water. The result of this step is the laking of the corpuscles and liberation of the hemoglobin. These tubes are immediately centrifuged at high speed, and the supernatant fluid subsequently pipetted off with a sterile pipet attached to a water suction pump.

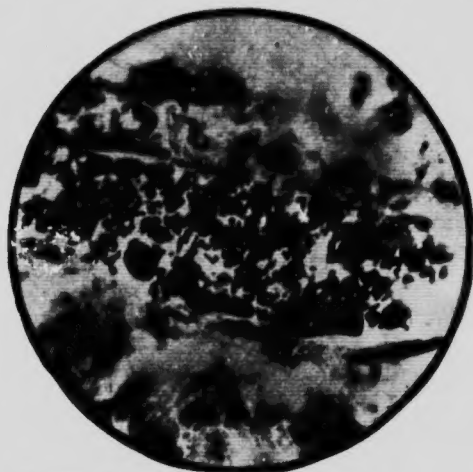


Fig. 1 (R. 517).—Bacteria growing in blood vessel of the myocardium.

The sediment remaining in the bottom of the tubes is composed of the broken down hulls of the corpuscles together with any bacteria which may be present. This operation is performed inside a glass cabinet which contains a Bunsen burner, the tube to the suction pump and the tap from the broth reservoir. By merely adding bouillon to the sediment, each centrifuge tube is converted into a culture flask, and is now ready to be placed in the incubator. The sediment of two tubes is reserved to be mixed with ascitic agar for anaerobic conditions. This is obtained in a satisfactory manner by making this mixture after the agar has

cooled to 40 C. and pouring into a tall test tube, the result being almost complete anaerobiosis at the bottom and a varying oxygen gradient as the top is approached.

The disadvantages of this method of blood culture are probably confined to two: The first is the time and skill required to carry out the technic, and this precludes its use as a routine measure. Such an objection, however, is not valid in a research laboratory. The second disadvantage is the large amount of unavoidable manipulation. Our only answer to that is the



Fig. 2 (R. 968)—Valvular vegetation.

absence of contamination, which has given us the confidence we have in our results.

The advantages are three: First of all, it seems reasonable that in a case in which the number of bacteria in the blood are few, the larger the sample taken, the more chance of obtaining the organism. Secondly, in chronic cases, such as we are investigating, it is believed that the patient has developed a certain amount of immune bodies in his serum. The provision in this method for the discarding of this immune serum will insure the organism having as little as possible to contend with. The serum removed is replaced by

ascitic fluid from a patient who may reasonably be considered not to have an immunity to a streptococcus infection. In this way our medium is enriched by the necessary serum proteins without the danger of including inhibiting bodies. Lastly, the hemoglobin, which is said to have a detrimental effect on certain strains of streptococci, is largely removed.

TABLE 1.—ACTION OF DIFFERENT STRAINS OF STREPTOCOCCUS VIRIDANS ON VARIOUS SUGAR MEDIUMS

Strain	Lac-tose	Levu-lose	Sac-charose	Mal-tose	Inu-lin	Ram-nose	Man-nit	As-par-agin	Sal-icin
R 15-15.....	+	+	—	+	—	—	—	—	—
R 15-14.....	+	+	+	+	—	—	—	—	—
R 15-55.....	+	+	—	+	—	—	—	—	—
R 15-11.....	+	+	+	+	—	+	—	—	+
R 15-12.....	+	+	+	+	—	—	—	—	+
R 15-50.....	+	+	+	+	—	—	—	—	+
R 15-F.....	+	—	+	+	—	—	—	—	—
R 15-Y.....	+	+	+	+	—	—	—	—	—
R 15-B.....	+	+	+	+	—	+	—	—	+
R 15-P.....	+	+	+	+	—	—	—	—	—
R 15-A.....	+	+	+	+	—	—	—	—	+
R 16-131.....	+	+	—	+	—	+	—	—	—

The organisms used in this series of experiments were obtained from blood in cases of subacute and chronic infectious endocarditis. All of the strains belong to the family of *Streptococcus viridans*, that is to say, while not all of them produced a distinct green on blood agar they resembled each other in their action on the various sugar mediums, and in their low grade of virulence. The minority, which were not green producing, yielded grayish fairly adherent colonies on blood agar; a few produced a brown color. All grades of chain formation, from very short to quite long forms, were found among them. They were usually arranged in pairs throughout the chain. They did not dissolve in bile salts. None of them fermented inulin. All produced acid and coagulation in lactose, maltose and levulose serum water. All but two acted in the same manner on saccharose. Fifty per cent. fermented salicin. Mannite and asparagin, as well as inulin, remained unchanged. The organisms are difficult to keep in stock, requiring to be transferred every two or three days. This is due to the inhibitory action of the acid which they produce on the glucose broth or agar used, the acidity going as high as 3 per cent., whereas

the reaction of the mediums on which they are planted should not be higher than $+0.5$. Latterly we have been making up the broth for stock cultures with 1 per cent. calcium carbonate, which insures the neutrality of the medium, and on this the organism lives for about seven days.

The animal experiments have been confined to an attempt to produce a condition in the rabbit analogous to that of the patient from whom the organism was obtained. The great difficulty of producing endocar-

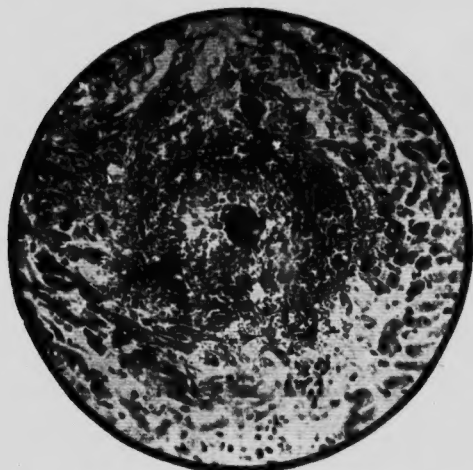


Fig. 3 (R. 664).—Focal lesion in myocardium. Note blood vessel plugged with bacteria.

ditis experimentally, without previous injury to the valves, is well known. After unsuccessful attempts had been made, several observers succeeded in producing the condition by adding to the emulsion of organisms injected, a mechanical irritant. Among those investigators were Hibbert,³ who added potato particles, Orth and Wyssowitsch,⁴ and Fulci,⁵ who used pulverized charcoal. These foreign bodies produced a condition of erosion on the valve cusps, which there-

3. Hibbert: *Fortschr. d. Med.*, 1886, iv, 1.

4. Orth and Wyssowitsch: *Virchows Arch. f. path. Anat.*, 1886, ciii, 300, 333.

5. Fulci: *Beitr. z. path. Anat.*, 1908, xliv, 349.

upon became a favorable soil for the inflammation of the organism. Lissauer,⁶ Horder,⁷ Rosenow⁸ and others have succeeded in producing endocarditis in rabbits without the aid of foreign irritants. The condition, of course, is produced with less difficulty with organisms of definite virulence. All are agreed on the fact that the streptococcus obtained in chronic infectious endocarditis is of very low virulence. Rosenow lays especial emphasis on this point. Believing that the strains of streptococci with which we were working were even less virulent than those which this author described, we were anxious to establish conclusive evidence of their ability to produce the disease in animals. Not only that, but we were equally anxious to learn whether these organisms of almost negligible virulence had nevertheless a special affinity for heart valves and the myocardium. We feel that the results of our investigations have justified this belief.

Rabbits from 6 weeks to 21½ months old were used. The majority were Belgian hares. The cultures were grown in vaccine bottles of 60 c.c. capacity, containing about 40 c.c. of ascitic glucose bouillon. A copious growth was obtained in twenty-four hours, and the bottle was then centrifuged at high speed in a special trunnion cup, and the supernatant bouillon pipetted off. This operation can be done with such nicety that practically no free fluid is left with the bacteria, which are thereupon suspended in 2 or 3 c.c. of sterile saline solution and injected intravenously into the rabbit. Filling the syringe with the emulsion and expelling it through a small needle leads to the clumps being largely broken up. This is repeated several times, and a uniform emulsion results. The marginal vein of the ear was used invariably. Each strain of organism is studied first of all in regard to its virulence. The lethal intravenous dose is roughly estimated by injecting varying amounts of the emulsion into several rabbits. The average dose tolerated is the amount of emulsion obtainable from one to two vaccine bottles. This is an enormous dose of bacteria to be introduced directly into the blood stream of a rabbit weighing, say, 700 grams. When the lethal dose and the *dosis tolerata*

6. Lissauer: *Centralbl. f. allg. Path. u. path. Anat.*, 1912, xxiii, 243.

7. Horder: *Quart. Jour. Med.*, 1902, ii, 289.

8. Rosenow, E. C.: *Jour. Infect. Dis.*, 1909, vi, 245; 1910, vii, 411, 429; 1912, p. 210; *Elective Localization of Streptococci*, *THE JOURNAL A. M. A.*, Nov. 13, 1915, p. 1687.

are established, we try to keep the injections up to the highest point possible without killing the animal too quickly.

In Table 2, only a few of the cultures obtained are represented. It is impossible to give in this space the details relating to so many different strains. Those given, however, have been carefully chosen so as to be as representative as possible, and may be supplemented, if desired, by our records in the laboratory. Examples are described as showing a gradation of lesions — from ordinary streptococic bacteremia with

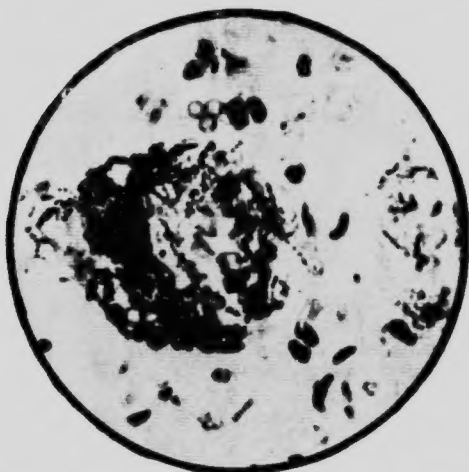


Fig. 4 (R. 574).—Bacteria growing in blood vessel of kidney.

out localization to endocarditis of the malignant, ulcerative type. Cases R 518, R 521, R 516, R 908, R 341 and R 665 are, in the order named, examples of just such a thing. Cases R 125 and R 281 are good examples of pericarditis in the absence of endocarditis. There seems to be first of all an affinity for the heart valves and, failing this, the organism seems to pick out the pericardium, or, in a few instances, the myocardium. R 991 is a case illustrative of the latter. Of all the rabbits coming to necropsy, heart lesions were found in 56.6 per cent. Endocarditis was present in 40 per cent., pericarditis in 13.3 per cent., myo-

carditis in 6 per cent., and aortitis in 3 per cent., while 30 per cent. gave a negative result. A slight degree of fever was present in all but one of the cases in which the temperature was taken. The average temperature of the rabbits with endocarditis was 103.8, the temperature of normal rabbits being about 102.5. These figures refer to readings by rectum. One case had a subnormal temperature—100.8. Blood cultures were taken during life in a number of cases, but although the results are interesting they are necessarily inconclusive, since the measure was not carried out as a routine. It would seem, however, that, excepting in the later stages of the disease, it is fairly difficult to produce a constant bacteremia with the organisms under discussion.

With this data before us, we feel that we have successfully demonstrated that while the organisms recovered in clinical cases of chronic infections of endocarditis are of an exceedingly low grade of virulence, they are nevertheless capable of producing in the rabbit not only a condition closely resembling that of the patient from which they were obtained, but also a condition which often ends fatally, accompanied by the usual signs of acute ulcerative, or so-called malignant endocarditis. We are therefore inclined to believe with Rosenow⁹ that the ordinary *Streptococcus viridans* or "endocarditis coccus" may produce all grades of endocarditis, and every grade may be produced in animals by the different strains of the organism. We also have shown that every grade of endocarditis may be produced in different animals by the same strain of organism.

Some evidence is at hand supporting the theory of implantation. A rabbit, R 374, inoculated with Strain R. 15-131 presented at necropsy a verrucose vegetation on the free edge of the valve. The microscopic examination of this specimen reveals no signs of capillary connection with this vegetation, and gives every appearance of its being merely an implantation on the edge of a valve which has been subjected to the constant erosive action of the intimate but intermittent pressure of its fellow. The fact that in the same rabbit there is found a similar vegetation at the base of the valve, or on the valve ring, does not necessarily

⁹ Rosenow, E. C.: Experimental Endocarditis, Jour. Infect. Dis., September, 1912.

TABLE 2.—ACTION OF STRAINS ON RABBITS

No. of Rabbit	Strain of Organism	No. and Amount of Intra-venous Injection	Clinical Picture	Death After First Inoculation	Postmortem Findings	Postmortem Cultures	Cultures from spleen	Cultures from liver and heart's
R. 518	Stein.....	2 medium 1 V. R. C.		18 days.....	Liver, spleen and kidney congested.....			

No. of Rabbit	Strain of organism	of Intra-venous Injection	Clinical Picture	Death After First Inoculation	Postmortem Findings	Postmortem Cultures
R. 518	Stein	2 medium 1 V. B. C.	7 days after last injection, rabbit killed, as it was getting very ill.	18 days	Liver, spleen and kidney congested.	Cultures from liver, spleen and heart's blood pure streptococci.
R. 517	Stein	3 medium doses	Died 15 min. after last injection	11 days	Heart: Subendothelial hemorrhage in muscle wall and also hemorrhage in one of the papillary muscles of the tricuspid valve; hemorrhage into the coronary arteries; very few fine vegetations along the free margin; heart muscle shows fatty degeneration; scattered throughout are bacterial emboli, some vessels occluded by bacterial emboli or thrombi of bacteria and corpuscles; nodules of round cell infiltration in mitral valve.	Cultures from liver, spleen and heart's blood gave streptococci.
R. 514	1596	3 medium doses	Last dose was 2 V. B. C.; died in 10 minutes; appeared in good health up to last injection	15 days	Heart: Small hemorrhage in one of the papillary muscles of mitral valve.	Cultures from liver, spleen and heart's blood gave streptococci.
R. 516	1596	2 small doses; 1 very large	Last dose was 2 V. B. C.; died in 10 minutes; appeared in good health up to last injection	15 days	Small vegetation on free margin of tricuspid valve; hemorrhage into papillary muscle of mitral valve.	Cultures from liver, spleen and heart's blood gave streptococci.
R. 513	R. T.	1 V. B. C.	Died in 10 minutes.	Ten min.	Hemorrhage into papillary muscle of right tricuspid valve.	No report
R. 605	R. T.	2 V. B. C.	Temp. of 102.4 three days in succession	11 days	Right tricuspid valve shows large verrucose vegetation, involving cusps; size of a small bean; photo taken.	Cultures: Heart's blood, spleen, liver and pericardium all negative.
R. 611	R. T.	4 V. B. C.	Temp.: maximum, 104.6; minimum, 102.4	15 days	Heart: Three large vegetations on tricuspid valve, white diameter from 3 to 5 mm.; lumen of valve almost completely closed; right auricle dilated; evidence of thrombosis in mitral valve; endocardium, hyaline, granular; kidneys congested, liver congested; mesenteric veins swollen.	Cultures from heart's blood, lungs, liver, pericardial cavity, and spleen all negative.
R. 67808	15131	1 V. B. C.	Died during night	A few hours	Pericarditis with effusion. Subserous hemorrhage about 1 cm. in diameter from pylorus; section of pericardium shows a hemorrhagic infarction; lungs congested; kidneys congested; liver congested; mesenteric veins swollen.	Cultures from heart's blood, lungs, liver, pericardial cavity, and spleen all negative.
R. 553	1596	2 small, 1 large, 1 medium	Rabbit very emaciated and weak	40 days	Heart: Small hemorrhage into tip of papillary muscle and mitral valve; small atheromatous patch in aortic arch just above aortic valve; 2 small areas of R. C. infiltration on the surface of one of the valves; some of the free margin and the base of the valve; areas of granular degeneration of heart muscle.	Cultures from liver, streptococci.
R. 366	R. T.	1 V. B. C. 1 V. P. C.	Rabbit became very thin	10 days	Heart: Pericarditis; endocarditis; large, white vegetations on tricuspid valve, about the size of a pea, pink verrucose. Vegetations stippled, corded, tendinous fibrous and granular. Numerous white patches on tricuspid valve, pericardial surface of the heart dull grayish white, other of the apparently normal.	Cultures from heart's blood and heart's blood gave streptococci.
R. 51	A. 561A 150	First culture from heart's blood and pericardium inoculated with strain 561A		5 hours	Heart: Pericarditis; endocarditis; large, white vegetations on tricuspid valve, about the size of a pea, pink verrucose. Vegetations stippled, corded, tendinous fibrous and granular. Numerous white patches on tricuspid valve, pericardial surface of the heart dull grayish white, other of the apparently normal.	Cultures from heart's blood, spleen and liver gave streptococci.
R. 508	1596	10 small doses; 1 medium; 1 large	Died during night after last dose	40 days	Hemorrhage into base of the closed aortic valve; early pericarditis; heart section shows vegetation about size of a pea.	Cultures from heart's blood and pericardial cavity gave streptococci.
R. 600	1596	10 small doses; 1 medium; 1 large	After last dose, rabbit very ataxic, died on the second day; culture from blood, spleen and liver gave streptococci.	10 days	Hemorrhage in coronal and mitral valves; tiny vegetations near base of mitral valve; no other normal.	No report
R. 115	1555	2 small doses		7 days	No evidence of endocarditis; liver congested; hydropneumothorax; pericarditis.	Cultures from heart's blood and pericardial cavity gave streptococci.
R. 601	1555 from R. 125	10 small doses; 1 large dose	Last dose 2 V. B. C.; died early next morning	92 days	Heart muscles lumpy; heart muscle shows numerous small scattered areas of round cell infiltration; some perivascular infiltration; kidney for its size and diffuse nephritis; numerous described areas of round cell infiltration.	Cultures from heart's blood and lung show streptococci A 115.

TABLE 2. ACTION OF STRAINS FROM NORMAL MOUTHS ON RABBITS

No. of Rabbit	Strain of Organism	No. and Amount of Injections	Clinical Picture	Death After First Inoculation	Postmortem Findings	Postmortem Cultures
R 64	P.A.	2 V.B.C.	3 days later very sick; all on its belly with continually nodding its head; temp. 97° F.	3 days after inoculation (chloroformed)	Heart: Pericarditis, myocarditis; vegetations on the tricuspid valve, tip of the papillary muscle and on the wall of the right auricle; these show a small vegetation around them; one hairy artery 3 mm. above the valves.	Culture from the blood shows streptococci, culture from pericardial cavity sterile
R 7	M. from a throat	1 V.B.C.	3 days after first dose rabbit very weak; it asthmatic and systolic murmurs could be heard over the heart; fluid in heart bag; rabbit had a few hours after examination	Dead 7 days after first inoculation	Heart: Myocarditis; small vegetations on the tricuspid valve, one fibrous adhesions; none small vegetations on the wall of the right ventricle; a big mass of verrucose vegetation on the tricuspid valve and extending down to the papillary muscles; kidneys show numerous greenish areas marked myocarditis; one kidney heart attached to the base of left tricuspid valve; 2 other vegetations on the tricuspid valve.	Culture from heart's blood; streptococci; also cultures from spleen and liver show streptococci
R 25	M.L. from a throat	4 injections: 1 V.B.C. After first injection temp. rose to 104° F. and rabbit developed a dry tick	3 days	3 days	Heart: Myocarditis; one kidney heart attached to the base of left tricuspid valve; 2 other vegetations on the tricuspid valve.	Cultures: heart's blood and liver; Streptococcus viridans; pericardium sterile
R 7	P. from a throat	2 V.B.C.	After first injection temp. 104° F.	3 days	Heart: Myocarditis present; verrucose vegetation of the tricuspid valve, involving all the cusps and the cusps of the aortic valve; the cusps of the aortic valve.	Cultures: heart's blood and liver; Streptococcus viridans
R 1	P. from a throat	2 V.B.C.	3 days after first dose 104° F.	3 days	Heart: Myocarditis; one kidney heart attached to the base of left tricuspid valve; 2 other vegetations on the tricuspid valve.	Cultures of the heart's blood and liver yield Streptococcus viridans
R 1	P. from a throat	2 V.B.C.	3 days after first dose 104° F.	3 days	Heart: Myocarditis; one kidney heart attached to the base of left tricuspid valve; 2 other vegetations on the tricuspid valve.	No cultures taken as the animal died so soon after the last inoculation

disprove or even cast doubt on the implantation theory, especially in view of the fact that the site of the vegetation is on the auricular side of the valve. What does seem to favor the embolic theory, however, is the finding of hemorrhages in the valve curtains and under the mural endothelium, as the earliest evidence of the work of the bacteria, or bacterial emboli. Cultures of streptococci can be obtained from these areas, and it is quite likely that on this favorable medium they begin the process which results in the familiar vegetation.

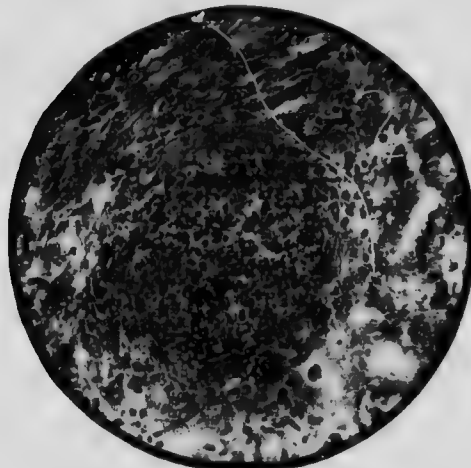


Fig. 5 (R. 574).—Focal lesion in kidney.

The clinical picture of the rabbit with chronic endocarditis resembles in a striking manner that of the patient with the same condition. The low grade of fever seems to be a constant feature of the disease, with periods of no fever, and bacteria-free blood stream. The fact that patients known to have endocarditis do not constantly yield positive blood cultures is, of course, well recognized. In the light of our findings there is no doubt in our minds that if we only had a way of knowing the opportune time, we might recover the streptococcus from the blood in many of these cases which now yield negative results. There is every reason to believe that the presence of strepto-

cocci in the blood does not necessarily mean, as was formerly supposed, a fatal issue. Rabbits from which we obtained positive blood cultures apparently, some in recovered cases, lived for months, and when chloroformed, yielded negative results at necropsy. Similarly, in clinical cases with bacteremia there has been improvement, and no doubt the animals will entirely recover. The loss in weight of the rabbits with endocarditis was another noticeable feature of the clinical picture, which also corresponds with the findings in the human types. In some cases the emaciation was quite marked and, at least to some degree, seems to be a constant feature in the animals.

It is exceedingly interesting to note the evident elective localization on the heart, and especially on the heart valve of these strains of streptococci, whose habitat was the heart valve and blood stream of their human host. Injected into the vein, they are pumped immediately into all parts of the body. Why do they not localize in the kidney, the appendix or the joints? Case R 281 was inoculated with a culture from the pericardial cavity of R 9780S with pericarditis. The necropsy next day revealed marked hemorrhages into the pericardium, and the pericardial surface of the heart was definitely inflamed. Yet the heart valves were perfectly normal. Surely there is something uncanny about the way in which these interesting organisms select their own peculiar scene of activity. Possibly much lies hidden in the expressions "elective localization" and "affinity," with which we dismiss these interesting phenomena from our discussions.

The similarity between the organisms recovered from the blood and the streptococci usually found in the tonsils and mouth of normal individuals is worthy of note. This is to be noted not only in the sugar reactions but also in animal experiments. Nor is this similarity at all surprising. If we believe, as we assert, that the *Streptococcus viridans* passes through the diseased tonsil, suppurating alveolar socket, or other favorable lesions of the buccal and pharyngeal cavities, and, entering the blood stream, localizes on the heart valves, is it to be wondered at that the similarity to the saprophytic relative still living in the mouth should be at least outstanding enough to be noticeable? We felt that it was altogether likely that these saprophytic streptococci living in the normal mouth could be

proved to have the power to produce endocarditis in an identical manner to that of the organisms described in the foregoing experiments, and we inaugurated plans to carry on at once the necessary experiments to determine this point.

Both Horder¹⁰ and Rosenow⁹ have reported the successful production of endocarditis in rabbits from streptococci isolated from normal saliva (Horder) and tonsils (Rosenow). Their experiments, however, were

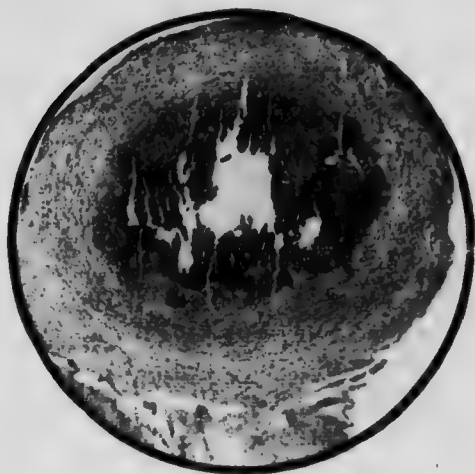


Fig. 6 (A. 374).—Section of vegetation.

very limited, and no attempt was made to issue accurate statistics. Our series, while larger, is not as comprehensive as we should have liked. Still, as far as it goes, it is quite conclusive. The cultures for this series of experiments were obtained from the teeth, gums and tonsils of medical students in the bacteriologic classroom. Eleven strains in all were used, and thirty-one rabbits were inoculated. The technic followed was identical with that used in the experiments recorded in the earlier part of the paper. All the strains were of the *viridans* variety of streptococcus.

10. Horder: Quart. Jour. Med., April, 1909; Local Gov. Rep., 1906-1907.

That is to say, they were capable of producing green on blood agar, gave typical fermentative reactions on the various sugars, and their other cultural characteristics were typical. They did not ferment inulin. Heart lesions of one kind or another were present in 80.6 per cent. of the rabbits. Of these, myocarditis came first with 67.7 per cent., and endocarditis next with 58 per cent. Aortitis was present in 32 per cent., and pericarditis was found in 22.5 per cent. Arthritis was demonstrated in 12.5 per cent.



Fig. 7 (R. 374).—Reaction about blood vessel just beneath endocardium, which was bulging at this point. Probably early stage of mural vegetation.

It will be seen (Table 3) that the percentage of positive results in connection with the mouth and throat series is greater than that of the series from strains recovered from the blood. This may be accounted for by the fact that subcultures were made of some of the latter several times before the first inoculation was made, whereas in the former, owing to increased facilities, we were able to begin inoculations from the first and second subculture, or as soon as pure culture was obtained. This discrepancy was true in only a few cases, however, and the presumption is

that the organisms, after reaching the blood, remain as avirulent as those which are left in the mouth.

The proportion of myocarditis is high, and evidently no higher than it should be. Although cloudy swelling and fatty degeneration were counted as myocarditis, the great majority had also focal areas of infiltration with round cells, and, less frequently, with polymorphonuclear cells. There were a great number of sections made in those cases showing on casual examination no focal lesions, and careful study given them. It seems likely, from our findings, that the presence of these focal areas would be demonstrated in some hitherto considered negative.

The fact that none of the strains isolated from the blood in cases of endocarditis produced joint lesions.

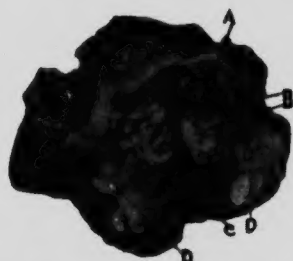


Fig. 8 (R. 341).—A, right auricle; B, vegetations on tricuspid valves; C, right ventricle; D, D, cut surfaces of same vegetation.

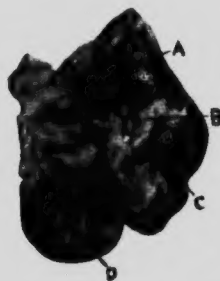


Fig. 9 (R. 665).—A, right auricle; B, vegetations on valve cusps; C, right ventricle; D, left ventricle.

whereas definite arthritis was present in four cases from strains isolated from the mouth and tonsils, seems significant to us. It strongly suggests two possible explanations: First, that an organism may have in some instances a dual affinity, and depending on environment and conditions existing in the particular individual in which they are present, they may attack one organ, or another, or both. Second, and more likely, that two types were present in one culture and each type produced its respective lesion. Pure cultures only of *Streptococcus viridans* were sought, and on being obtained, no attempt was made to grow the organism from one colony alone. When we speak of dual affinity, the term must of course be taken in its

broad application. We all know that practically every pathogenic organism can attack a variety of structures. That is not what is meant. But just as the pneumococcus has a special predilection for the respiratory tract, though it does produce lesions elsewhere, and in the same way the meningococcus for the meninges, so the *Streptococcus viridans* confines itself to the heart, or to the joints, or, as we suggested above, to both. It seems plain from our findings, at any rate, that the type of strain in the mouth and throat has a great deal to do with the type of lesion the patient is liable to develop. Take for example the results in respect to aortitis. The regularity with which this condition, in conjunction with endocarditis, was produced by certain strains is very striking. Strains L-7 and M-8 yielded aortitis in every instance. Strain M-8 was inoculated into six rabbits, and definite aortitis resulted in all.

Although the *Streptococcus viridans* usually produces chronic lesions, in which round cells greatly predominate, it can attract leukocytes in relatively large numbers, and therefore produce microscopic abscesses. Sections of vegetations show a small area in the center, composed chiefly of polymorphonuclear leukocytes with some of the nuclei undergoing fragmentation. There are also present some lymphocytes. Surrounding this focal mass of cells there is an area of proliferating endothelial cells. Some of the older vegetations show fatty changes with calcareous deposits in the center.

The focal areas in the heart muscle generally surround a small blood vessel which is completely occluded by a bacterial embolus. There is a necrosis of the muscle fibers, and a round cell infiltration composed of both polymorphonuclear leukocytes and lymphocytes.

CONCLUSION

Let us venture to suggest that we have established three important points:

1. The *Streptococcus viridans*, isolated from the blood in cases of chronic infectious endocarditis, is of very low virulence, probably lower than any hitherto reported as being recovered from a similar source.

2. These streptococci are capable of producing lesions

in animals identical to those found in the patients from whose blood the organisms were obtained.

3. The strains of *Streptococcus viridans* isolated from the mouth of normal individuals are similar to those isolated from the blood of patients suffering from chronic endocarditis, and are equally capable of producing heart lesions in the rabbit.

UNIVERSITY OF TORONTO STUDIES
MEDICAL RESEARCH FUND

No. 1: On the haemolytic properties of fatty acids and their relation to the causation of toxic haemolysis and pernicious anaemia, by W. F. MCPHEDRAN	
No. 2: Biological curves obtained during the onset and course of tuberculous infection, by A. H. CAULFIELD AND F. S. MINNS	
No. 3: Tuberculin treatment based upon clinical and biological data, by A. H. CAULFIELD AND F. S. MINNS	1.00
No. 4: The excretion of nitrogen in fever, by N. C. SHARPE and K. M. B. SIMON	
No. 5: On fatty changes in the liver, heart and kidney, by C. G. IMRIE	
No. 6: On the fat in the blood in a case of lipaemia, by C. G. IMRIE	
No. 7: A spectroscopic examination of the colour reactions of certain indol derivatives and of the urine of dogs after their administration, by ANNIE HOMER	
No. 8: A method for the estimation of the tryptophane contents of proteins, involving the use of baryta as a hydrolyzing agent, by ANNIE HOMER	1.00
No. 9: The relation between the administration of tryptophane to dogs and the elimination of kynurenic acid in their urine, by ANNIE HOMER	
No. 10: A suggestion as to the cause of the lessened production of indol in media containing glucose, by ANNIE HOMER	0.25
No. 11: Experimental endocarditis, by H. K. DETWEILER and W. L. ROBINSON	0.25